Effect of menstrual cycle phase on the concentration of individual carotenoids in lipoproteins of premenopausal women: a controlled dietary study[1,2]

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ABSTRACT   Because premenopausal women experience cyclic fluctuations of plasma carotenoids and their lipoprotein carriers, it is hypothesized that carotenoid concentrations in lipoprotein fractions fluctuate by phase of the menstrual cycle. Nine women ate a standard set of carotenoid-rich foods daily for two cycles under isoenergetic conditions. In the second cycle, hormones and carotenoids in lipoprotein fractions were measured in the early and late follicular and luteal phases. α-Carotene concentrations in the LDL fraction were lower in the early than in the late follicular phase ($P = 0.03$) on the basis of regression analysis. β-carotene concentrations in the LDL fraction and the HDL_2 subfraction were higher in the late follicular than in the luteal phase ($P = 0.02$ and $P = 0.04$, respectively). Lutein/zeaxanthin concentrations in the LDL and HDL fractions were higher in the late follicular than in the luteal phase ($P = 0.03$ and $P = 0.02$, respectively). In each phase, 80% of α-carotene, 82% of β-carotene, 85% of lycopene, and 64% of lutein/zeaxanthin were distributed in the LDL fraction. Among the hydrocarbon cartenoids, 18% of α-carotene and of β-carotene and 13% of lycopene were distributed in the HDL fraction, with slightly more in the HDL_2 than in the HDL_3 subfraction. In contrast 34% of lutein/zeaxanthin was distributed in the HDL fraction with more concentrated in the HDL_3.than in the HDL_2 subfraction. Less than 4% of any carotenoid was found in the VLDL+IDL (intermediate-density-lipoprotein) fractions. Thus, the hydrocarbon carotenoids were highly concentrated in the LDL fraction and xanthophyll was more evenly distributed in the LDL and HDL fractions. The cyclic fluctuations of these carotenoids in lipoprotein fractions add another dimension to the understanding of their transport and physiologic function. *Am J Clin Nutr* 1998;67:81–7.

KEY WORDS   Carotenoids; lipoproteins; menstrual cycle; premenopausal women; controlled diet; Carotenoid, Lipids, Hormones Study

INTRODUCTION

Dietary intakes of carotenoid-rich fruit and vegetables as well as of individual carotenoids are inversely associated with the risk of lung, breast, and prostate cancers (1–4). In contrast, randomized clinical trials of pharmaceutical doses of β-carotene have shown either no effect or an increased risk of lung cancer (5–7).

Therefore, future nutrition and cancer prevention studies are likely to use whole foods increasingly rather than supplements.

Apart from β-carotene, fundamental aspects of the absorption and transport of other dietary carotenoids (α-carotene, lycopene, cryptoxanthin, and lutein/zeaxanthin) and their metabolites are not as well understood (8–11). A complex array of factors potentially influences the process of absorption and transport of the carotenoids, including, for example, dietary fat and fiber intakes, vitamin A status, hormone status, and drug interactions (8). Yet, research has been characterized primarily by the plasma response to carotenoids from either a dietary source or a pharmaceutical dose as well as by descriptions of tissue concentrations of carotenoids (10–22).

Plasma carotenoids are transported in lipoproteins: α- and β-carotene and lycopene are transported primarily in LDL, whereas lutein/zeaxanthin is more equally distributed between HDLs and LDLs (15–17). Whereas early research on carotenoid transport used laboratory analyses that are no longer state of the art (15, 18), more recent studies have typically examined the distribution of β-carotene in lipoproteins after a single (19, 20) or long-term pharmaceutical dose of β-carotene (21, 22). One controlled dietary study (16) and one free-living study (17) examined the distributions of carotenoids in lipoprotein fractions. Most research has been conducted in men (16, 18, 19, 22) and two studies had insufficient numbers of women to stratify the analysis by sex (17, 20).

A study of the fluctuation of plasma carotenoids, lipoproteins, and serum hormones by phase of the menstrual cycle was conducted in 1992 in healthy, premenopausal women given a daily, standardized, carotenoid-rich diet for two cycles under the same controlled dietary conditions (23). Plasma carotenoid concentrations during the second cycle were lowest at menses. Plasma

lutein/zeaxanthin concentrations were higher at all three phases (early and late follicular and luteal phases) after menses and peaked at the late follicular and luteal phases. Plasma β-carotene concentrations peaked at the late follicular phase, whereas plasma lycopene concentrations peaked at the luteal phase. Plasma LDL concentrations were lower in the luteal than in the early follicular phase, and plasma HDL concentrations were higher in the late follicular phase than during menses (24). Because premenopausal women experience cyclic changes in the lipoprotein carriers of carotenoids (24–26) and in plasma carotenoid concentrations (23), it is expected that carotenoid concentrations in the lipoprotein fractions might also fluctuate by phase of the menstrual cycle. This hypothesis was examined by using data from the second cycle of a controlled dietary study known as the Carotenoids, Lipids, Hormones (CLH) Study (23).

SUBJECTS AND METHODS

Female volunteers were screened initially by phone and selected to participate after a physical and gynecologic exam, an in-person interview, and biochemistry tests at the National Institutes of Health Clinical Center (23). Participants met the following eligibility criteria: *1*) they were aged 20–34 y; *2*) they were nonsmokers; *3*) they were not pregnant, were not receiving hormone preparations, and had not breast-fed in the past 6 mo; *4*) they had no history or clinical signs of gynecologic problems; *5*) they were within 20% of their ideal weight-for-height based on age- and sex-specific reference values, because very thin and obese women experience anovulatory cycles more frequently than do women within the normal range (27); *6*) they had plasma cholesterol, triacylglycerol, and hemoglobin concentrations within the normal range for women of reproductive age, to eliminate individuals who had potential problems in lipid metabolism or who were anemic; *7*) they were not consuming a restricted diet nor regularly consuming vitamin-mineral supplements; and *8*) they were willing to stop taking supplements and to abstain from alcohol during the controlled dietary study (23). The protocol was approved by the institutional review boards of the George Washington University School of Medicine, the National Cancer Institute, and the US Department of Agriculture (USDA) Human Studies Review Committee.

Of the 14 women who began the controlled dietary study, 5 were removed from data analysis because 1 experienced an anovulatory cycle, 1 other did not show a plasma carotenoid response to the diet (23), and 3 had their plasma lipoprotein specimens stored under inappropriate conditions (4 °C) for carotenoid analysis. Therefore, data analysis was restricted to the remaining nine women.

Study design

The study was designed as a free-living study of women during one menstrual cycle to confirm ovulation, followed by a controlled dietary study for two cycles. The controlled dietary study was conducted at the USDA Beltsville Human Nutrition Research Center (BHNRC) and consisted of a 7-d repeat menu cycle. The daily diet provided 36% of energy as fat, 19% as protein, and 55% as carbohydrates, with 34 kg fiber/kJ and a ratio of polyunsaturated to saturated fatty acids of 0.53. A standard set of carotenoid-rich foods (50 g peas, 16 g carrots, 61 g broccoli, and 48 g tomato sauce) was given in the same amount daily. The carotenoid-rich food sources provided 0.7 mg α-carotene/d, 2.5 mg β-carotene/d, 2.6 mg lutein/d, and 4.3 mg lycopene/d, for a total of 10 mg carotenoids/d (23). No other carotenoid-rich foods were eaten during the controlled dietary study. All meals were prepared at the BHNRC. The women ate their meals at the BHNRC dining facility, except for carryout lunches on weekdays and carryout meals on weekends and holidays. To maintain isoenergetic conditions, participants were weighed daily before breakfast and their energy intake was increased or decreased on an individual basis in increments of 47.6 kJ if they lost or gained ≥ 1 kg and maintained that weight for ≥ 3d.

Measurement of serum hormones, plasma lipids, and carotenoids in the lipoprotein fractions

Blood samples were collected between 0600 and 0700 from women who had fasted for > 12 h. Samples were collected into EDTA-containing evacuated tubes for carotenoid and lipoprotein analyses and into EDTA-free evacuated tubes for hormone analyses. Blood drawing for lipoprotein fractions and carotenoids occurred on the following days of the menstrual cycle: *1*) menses day 4 (ie, the early follicular phase); *2*) menses days 12, 13, 14, or 15 (ie, the late follicular phase), whichever day corresponded to the day of each woman's luteinizing hormone surge (LH) ($n = 7$) or the day after her LH surge ($n = 2$); and *3*) menses day 19, 20, 21, or 22 (ie, the midluteal phase), whichever day corresponded to 7 or 8 d after the LH surge. An ovulatory cycle was defined as a serum LH concentration > 30 U/L at the time of the LH surge and a serum progesterone concentration > 13 nmol/L at the midluteal phase.

Hormones were measured by the Immunoassay Laboratory of the Genetics and In Vitro Fertilization Institute, Fairfax, VA, on the same day that blood was drawn. Serum progesterone concentrations were measured by direct radioimmunoassay (Diagnostic Products Corp, La Jolla, CA). Serum LH was quantified with the monoclonal Immuno-Radio-Metric-Assay (Serono, Randolph, MA). Procedures followed the analytic methods described by Munabi et al (28).

Blood samples for lipoprotein fractionation and subsequent lipid analyses were covered with aluminum foil, placed on crushed ice, and delivered in a covered container to the George Washington University Lipid Research Clinic Laboratory before 1000 on the same day that blood was drawn. The samples were centrifuged at 1500 × *g* for 20 min at 4 °C to obtain plasma. The HDL fraction and HDL$_3$ subfraction were isolated by using the precipitation procedures of Gidez et al (29) and Muesing et al (30). Specifically, samples of plasma (3 mL) were placed on ice and treated with heparin and magnesium chloride at final concentrations of 206 kU/L (206 U/mL) and 92 mmol/L, respectively. After centrifugation, samples (1.5 mL) of the HDL supernatant fractions were placed in a water bath at 23 °C and dextran sulfate (molecular weight: 15 000) was added to a final concentration of 0.13%. Samples of the HDL$_3$ supernatant fractions obtained after centrifugation and of the HDL fractions were placed in cryogenic vials and placed at −70 °C until analyzed for lipids and carotenoids after receipt of the final samples of a participant.

Fractions of VLDL plus intermediate-density lipoprotein (VLDL+IDL) and LDL+HDL were isolated by preparative ultracentrifugation (100 000 × *g* at 10 °C for 18 h) essentially as described in the Lipid Research Clinic Program protocol (31). However, the nonprotein density of plasma (5 mL) was adjusted to 1.019 by adding 1.17 mL NaBr per 1 mmol EDTA/L (pH 7.0)

at a density of 1.063 kg/L (verified by pycnometry). The samples were centrifuged in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Fullerton, CA) at 10 °C for 18 h at 100 000 x g with a 50.3 rotor. The VLDL+IDL and LDL+HDL fractions were recovered quantitatively by tube-slicing at the approximate midpoint between the turbid VLDL+IDL fraction at the top of the tube and the orange-colored LDL band near the bottom of the tube (2.3 cm below the bottom of the tube cap). The VLDL+IDL and LDL+HDL fractions were each brought to a final volume of 5.0 mL. Centrifugation at a density of 1.019 kg/L allows for the determination of LDL constituents without the confounding factor of the IDL contribution. Recoveries of cholesterol ranged from 97% to 101%.

Preparation of the HDL fraction and HDL_3 subfraction and initiation of ultracentrifugation were performed on the day the samples were collected. All isolations were conducted with fluorescent lighting turned off and exposure to other light minimized. All solutions for lipoprotein fractionation were prepared in sufficient quantities to be used for the duration of the study, and all lipoprotein fraction procedures were carried out by the same person (RM).

As participants completed their final visit, their frozen samples were thawed, mixed thoroughly, and analyzed. To avoid day-to-day analytic variations, all samples of an individual were batched and analyzed sequentially as a set. Plasma cholesterol and triacylglycerol were analyzed enzymatically on Abbott VP analyzers (Abbott Laboratories, Chicago) with reagents and standards from Sigma Chemical Co, St Louis (30). CVs for analysis of cholesterol, triacylglycerol, and HDL-cholesterol controls were 1.5%, 1.8%, and 1.0%, respectively LDL/carotenoid concentrations were defined as the difference between LDL+HDL and HDL values, and HDL_2/carotenoid concentrations were defined as the difference between HDL and HDL_3 values. The laboratory maintains standardization with the Centers for Disease Control and Prevention for analysis of cholesterol, triacylglycerol, and HDL cholesterol.

Carotenoids were extracted by the Tufts-USDA Human Research Nutrition Center on Aging by using a 200-μL sample added to 0.5 mL saline. γ-Carotene in ethanol was added as an internal standard (Hoffmann-La Roche, Inc, Nutley, NJ). The mixture was extracted by using 2 mL chloroform:methanol (2:1, by vol). The mixture was vortexed and then centrifuged at 800 x g at 4 °C for 15 min. The chloroform layer was removed and evaporated to dryness under nitrogen. A second extraction was performed on the mixture by using 3 mL hexane. The mixture was vortexed and centrifuged as above. The hexane layer was combined with the first extraction and evaporated to dryness under nitrogen. The residue from the two extractions was redissolved in 150 μL ethanol, vortexed, and sonicated for 30 s.

A 50-μL aliquot was used for separation and quantification of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene by using reversed-phase HPLC (32). The HPLC system consisted of a Perkin-Elmer (Norwalk, CT) LC-410 pump, a Waters (Milford, MA) 994 programmable photodiode array detector set at 455 nm, and a Perkin-Elmer ISS-100 autosampler; the column was a C18 Pecosphere-3 (Perkin-Elmer). The HPLC solvents were acetonitrile, tetrahydrofuran, and water (50:20:30, by vol, and 1% ammonium acetate in water; solvent A) and acetonitrile, tetrahydrofuran, and water (50:44:6, by vol; solvent B) (JT Baker Chemical Co, Phillipsburg, NJ). The gradient was as follows: 100% solvent A was used for 3 min followed by a 7-min linear gradient to 83% solvent B, a 15-min hold at 83% solvent B, and then a 2-min gradient back to 100% solvent A. The limit of detection for β-carotene and lycopene was 0.2 pmol. The intra- and interassay CVs for β-carotene were 3.4% and 2.0%, respectively.

Statistical analysis

Analysis of variance (ANOVA) modeling was used to compare mean individual carotenoids in each lipoprotein fraction [VLDL+IDL; LDL; HDL; HDL_3; HDL_2 (ie, $HDL - HDL_3$)] by phase of the menstrual cycle. PROC GLM (SAS Institute, Cary, NC) was used for the analyses. The ANOVAs included a separate intercept (ie, fixed effect) for each person, to take into account the correlation among repeated measurements over the cycle for each person. An adjustment for VLDL+IDL and LDL carotenoid concentrations was added to the model to correct for the differences in plasma lipoprotein carotenoid concentrations in contrast with the actual plasma carotenoid concentrations. The sum of the lipoprotein concentrations ranged from 97% to 101% of the plasma concentrations. The least-squares-mean ($\pm$ SE) concentration of a carotenoid in a lipoprotein fraction by phase of the menstrual cycle was generated from each model. The percentage of a carotenoid in each lipoprotein was calculated by summing the least-squares-mean values for each carotenoid in VLDL+IDL, LDL, and HDL, then dividing each carotenoid in a specific lipoprotein by the sum and multiplying by 100. Findings were considered statistically significant when the two-sided P value was ≤ 0.05.

RESULTS

On average, the women who participated in the free-living study for one menstrual cycle followed by the controlled dietary study for two menstrual cycles were aged 28 y, had a high school education, and were slightly thinner than US women of reproductive age (33). Their average menstrual cycle length (for each study cycle) was 26 d (**Table 1**). Their characteristics were similar to those of the sample ($n = 12$) used for the analysis of the cyclic fluctuation of plasma carotenoids (23), and they had cyclic fluctuations that were comparable with those of the sample of nine in the present study (data not shown).

α-Carotene concentrations in the LDL fraction were lower in the early follicular than in the late follicular and luteal phases ($P = 0.02$; $P = 0.03$, respectively) (**Table 2**). In each phase, 79–80% of α-carotene was distributed in the LDL fraction; another 10% and 8% were in the HDL_2 and HDL_3 subfractions, respectively; and the remaining 2% was in the VLDL+IDL fraction.

β-Carotene concentrations in the LDL fraction peaked at the late follicular phase (early compared with the late follicular phase: $P =$

TABLE 1
Characteristics of the study participants in the Carotenoids, Lipids, Hormones Study[1]

Age (y)	28 ± 3
Height (cm)	167 ± 5
Weight (kg)	58 ± 7
BMI (kg/m²)	21 ± 2
Education (y)	12 ± 0.7
Menstrual cycle length (d)	26 ± 1

[1] $\bar{x} \pm$ SD; $n = 9$.

0.02; late follicular compared with the luteal phase: $P = 0.04$). β-Carotene concentrations in the HDL_2 subfraction were higher in the late follicular than in the luteal phase ($P = 0.04$). In each phase, 81–82% of β-carotene was distributed in the LDL fraction; another 8–9% and 7–8% were in the HDL_2 and HDL_3 subfractions, respectively; and the remaining 2–3% was in the VLDL+IDL subfraction.

Lutein/zeaxanthin concentrations in the LDL and HDL fractions were significantly higher in the late follicular than in the luteal phase ($P = 0.03$ for LDL; $P = 0.02$ for HDL) whereas its concentration in the VLDL+IDL fraction was lower in the late follicular than in the luteal phase ($P = 0.03$). In each phase, 63–64% was distributed in the LDL fraction, 34% was in the HDL fraction, and the remaining 3–4% was in the VLDL+IDL fraction. Cyclic fluctuation of the HDL subfractions revealed that 23%, 18%, and 22% was in the HDL_3 subfraction and 11%, 16%, and 12% was in the HDL_2 subfraction during the early follicular, late follicular, and luteal phases, respectively; however, changes in the HDL subfractions were not significantly different.

Concentrations of lycopene in lipoprotein fractions did not differ by phase of the cycle, except that concentrations in VLDL+IDL were lower in the late follicular than in the luteal phase ($P = 0.006$). In each phase, 84–85% of lycopene was distributed in the LDL fraction; 7% and 5–6% were in the HDL_2 and HDL_3 subfractions, respectively; and 3–4% was in the VLDL+IDL fraction. Finally, because cryptoxanthin-rich foods were not consumed during this controlled dietary study, the concentrations of β-cryptoxanthin in lipoproteins are not presented. None of the lipoproteins varied by phase of the cycle, except for the HDL_2 subfraction, which was higher in the late follicular than in the early follicular phase ($P = 0.03$).

DISCUSSION

The purpose of this study was to examine the distribution of individual carotenoids in lipoprotein fractions by phase of the menstrual cycle under controlled dietary conditions. The cyclic fluctuation of carotenoids in specific lipoprotein fractions differed by carotenoid. Whereas α-carotene concentrations in the LDL fraction were lower in the early follicular than in the late follicular and luteal phases, β-carotene concentrations in the LDL fraction and in the HDL_2 subfraction were higher in the late follicular than in the luteal phase. Lutein/zeaxanthin concentrations in the LDL and HDL fractions were higher in the late follicular than in the luteal phase. Finally, changes in VLDL+IDL occurred, but concentrations of each carotenoid in these fractions were insignificant and therefore not considered of biological import.

The specificity of the carotenoid-lipoprotein relation by phase of the menstrual cycle was examined when plasma carotenoid concentrations reached steady state in the second cycle of the controlled dietary study (23). The potential physiologic effect of these findings can be described in the following illustration using β-carotene. As indicated in Table 2, the concentrations of β-carotene in each lipoprotein fraction increased from the early to late follicular phase and decreased (except for HDL_3, which essentially remained the same) from the late follicular to the luteal phase. Because there was a fairly equivalent cyclic increase and decrease in each lipoprotein, it was not apparent that β-carotene was transferred from one lipoprotein fraction to another, in contrast with the findings of a pharmacokinetics study of a single dose of β-carotene in men (19).

The implications of these findings for ovarian function should be discussed in the context of peak plasma LDL-cholesterol concentrations during the early follicular phase followed by peak plasma β-carotene concentrations in the late follicular phase (23, 24). LDL cholesterol is required for progesterone biosynthesis (34). The cyclic fluctuation of β-carotene in the LDL fraction from the early to the late follicular phase correlated with the peak progesterone concentration at the midluteal phase (Spearman $r = 0.77$, $P = 0.03$), with a similar correlation between cyclic changes in plasma β-carotene concentrations and the peak progesterone concentration (Spearman $r = 0.86$, $P = 0.0004$). These relations might be driven by the LH surge at ovulation. LH causes an increase in the number of LDL receptor sites in the corpus luteum during the midluteal phase and is responsible for the acute and long-term regulation of progesterone biosynthesis (35). Its acute effect is on the mobilization of LDL, whereas the long-term effect is on the enzymes responsible for conversion of cholesterol into progesterone. LDL is taken up by corpus luteum tissue via the receptors and undergoes metabolism (35). At the same time, β-carotene in the LDL fraction may be deposited in the ovaries, which would correspond to the reported high concentration of β-carotene in the corpus luteum of human ovaries (36–38).

The potential effects of β-carotene on ovarian function are described in both in vitro and in vivo studies (39, 40). After incubation of pig luteal cells with either β-carotene or retinoic acid, and in the presence of LH, progesterone in the medium increased 10-fold with β-carotene in contrast with a 5-fold increase with retinoic acid (39). Dairy cows that had consumed a diet low in β-carotene and had plasma β-carotene concentrations ≤ 930 nmol/L (50 mg/dL) had significantly increased fertility after β-carotene supplementation compared with controls (40). Therefore β-carotene might play a role in ovarian steroidogenesis and fertility; however, future research is needed on the LDL receptor, how β-carotene is deposited in the cell, and how β-carotene functions in progesterone secretion. Research on the role of β-carotene in ovarian function needs to be conducted in subjects who absorb carotenoids rather than in those who do not, as done previously in animals containing white fat.

In each phase of the second cycle, 79–81% of α-carotene and of β-carotene, 84–5% of lycopene, and 63–4% of lutein/zeaxanthin were distributed in the LDL fraction. Up to 18% of α-carotene and of β-carotene and 13% of lycopene were distributed in the HDL fraction, whereas 34% of lutein/zeaxanthin was distributed in the HDL fraction. Therefore, there was a relatively constant percentage of the carotenoids in the LDL fraction over the cycle, whereas percentages in the HDL subfractions did not vary significantly by phase of the cycle.

In earlier research, 67–80% of β-carotene was distributed in the LDL fraction and 15–23% was distributed in the HDL fraction (16–22). Of the other carotenoids, 73%–77% of lycopene was distributed in the LDL fraction, 16–17% was in the HDL fraction, and 10–12% was in the VLDL fraction. For lutein/zeaxanthin, 31–47% was distributed in the LDL fraction, 42–53% was in the HDL fraction, and 10–20% was in the VLDL+IDL fraction (16, 17, 21). Although percentages of the hydrocarbon carotenoids were lower in the LDL and higher in the HDL fractions in earlier research than in the present study, percentages in the CLH Study were within 1 SD of those provided by Romanchik et al (17). The major differences appeared in the percentage of lutein/zeaxanthin in the LDL and HDL fractions and in the percentage of all the carotenoids in VLDL+IDL fraction.

TABLE 2
Concentrations of α-carotene, β-carotene, lutein/zeaxanthin, and lycopene in the lipoprotein fractions and concentrations of lipoproteins by phase of the menstrual cycle: the Carotenoids, Lipids, Hormones Study[1]

	Early follicular phase	Late follicular phase	Luteal phase
α-Carotene (nmol/L)			
VLDL ± IDL	3.7 ± 0.04	2.2 ± 0.04	2.6 ± 0.03
LDL	96.2 ± 0.15	107.1 ± 0.15[2]	106.0 ± 0.12[2]
HDL$_2$	12.6 ± 0.05	12.6 ± 0.06	12.4 ± 0.05
HDL$_3$	9.6 ± 0.04	10.6 ± 0.04	10.2 ± 0.04
HDL	22.2 ± 0.04	23.1 ± 0.05	22.6 ± 0.04
β-Carotene (nmol/L)			
VLDL ± IDL	14.9 ± 1.5	15.4 ± 1.5	18.0 ± 1.1
LDL	466.7 ± 14.0	520.4 ± 14.0[2]	478.0 ± 11.3[3]
HDL$_2$	51.9 ± 2.6	57.3 ± 3.0	48.4 ± 2.4[3]
HDL$_3$	43.3 ± 2.1	45.0 ± 2.4	45.2 ± 2.1
HDL	95.2 ± 3.0	102.3 ± 3.5	93.6 ± 2.8
Lutein/zeaxanthin (nmol/L)			
VLDL ± IDL	13.4 ± 1.2	13.0 ± 1.2	16.6 ± 0.9[3]
LDL	292.7 ± 8.6	311.5 ± 8.6	283.7 ± 6.9[3]
HDL$_2$	52.6 ± 9.0	76.6 ± 10.7	54.0 ± 8.8
HDL$_3$	107.4 ± 7.9	87.5 ± 9.5	97.9 ± 7.7
HDL	160.0 ± 3.0	164.0 ± 3.5	152.1 ± 2.8[3]
Lycopene (nmol/L)			
VLDL ± IDL	27.9 ± 2.8	22.5 ± 2.6	33.9 ± 2.1[3]
LDL	672.2 ± 28.8	728.6 ± 28.8	709.4 ± 23.4
HDL$_2$	58.4 ± 3.3	62.3 ± 4.1	56.0 ± 3.3
HDL$_3$	45.0 ± 2.8	44.3 ± 3.3	47.4 ± 2.6
HDL	103.6 ± 4.7	106.6 ± 5.6	103.2 ± 4.5
Lipoprotein cholesterol (mmol/L)			
LDL	2.7 ± 0.06	2.7 ± 0.07	2.6 ± 0.06
HDL$_2$	0.5 ± 003	0.6 ± 0.03[2]	0.6 ± 0.02
HDL$_3$	0.8 ± 0.03	0.8 ± 0.03	0.9 ± 0.02
HDL	1.4 ± 0.02	1.5 ± 0.03	1.5 ± 0.02

[1] Least-squares means ± SE were generated by ANOVA. With use of Bonferroni's adjustment to account for multiple comparisons and a two-tailed type I error of 0.05, a conservative cutoff for statistical significance would be $P < 0.0007$.

[2] Significantly different from early follicular phase, $P \leq 0.05$ (Student's t test subsequent to ANOVA).

[3] Significantly different from late follicular phase, $P \leq 0.05$ (Student's t test subsequent to ANOVA).

Several factors might explain the range in the percentage distribution of a carotenoid in each lipoprotein fraction in this compared with earlier studies. Most studies examined the plasma response to a pharmaceutical dose of β-carotene in the free-living state (18–22). Differences in the kinetics of a pharmaceutical dose compared with dietary carotenoid-rich foods might explain the range in findings. Research in subjects consuming self-selected diets might introduce the potential for recent dietary intake of carotenoids, alcohol, or fiber to alter the distribution of carotenoids in lipoprotein fractions. Another factor might relate to the timing of the ingestion of the carotenoid-rich foods. In the current study, the carotenoid-rich foods were eaten at dinnertime. In an earlier study, when lutein/zeaxanthin and β-carotene were given in a combined dose, these carotenoids interacted, affecting their intestinal absorption, metabolism, and serum clearance (41). Therefore, the percentage of lutein/zeaxanthin in the lipoprotein fractions in this study might differ from its percentage in earlier studies because of the interaction effects of the simultaneous consumption of the carotenoid-rich foods.

Another factor may have been differences in the triacylglycerol concentrations of CLH Study patients compared with those of subjects in other studies. The premenopausal women in the present study had lower triacylglycerol concentrations (24) than patients in any other study (17, 21, 42) because women of reproductive age have lower triacylglycerol and LDL-cholesterol concentrations than men or older women (24). Because the triacylglycerol concentrations of the women in the CLH Study were low (0.59 mmol/L), they contained a considerable proportion of LDL and HDL, in contrast with the findings of these other studies (17, 21, 42). Thus, the proportion of VLDL+IDL in the triacylglycerols was lower and similarly the percentage of each carotenoid in the VLDL fraction was lower in the triacylglycerols of the CLH Study population than in the subjects in other studies (16–22, 42). Thus, the low triacylglycerol concentrations in the CLH Study group might form the physiologic basis for the low percentages of carotenoids in the VLDL fraction. Finally, Vogel et al (42) found comparable distributions of hydrocarbon carotenoids in lipoprotein fractions that were prepared by ultracentrifugation rather than by precipitation, but a higher concentration of lutein/zeaxanthin in the HDL fraction that was prepared by precipitation. Because HDL was quantified by precipitation in the CLH Study, concentrations of lutein/zeaxanthin in HDL would be expected to be higher than concentrations quantified by ultracentifugation. In fact, the percentage of lutein/zeaxanthin in the HDL fraction in the present study was lower than the percentage in the HDL fraction found in other studies, thereby reducing the probability that our

findings differ from the results of others because of different ana-
lytic laboratory techniques.

As in earlier research (16–22), the results of this analysis
should be interpreted with caution because of the small sample
size and because the levels of significance were not adjusted for
multiple comparisons. Also, one blood draw per phase increased
intraindividual variability in the estimate of the concentration of
a carotenoid in the lipoprotein fraction and tended to lower the
power to detect significant differences. Further investigation of
the cyclic fluctuation of the carotenoids in lipoproteins is war-
ranted in a larger sample population and blood specimens should
be collected during menses.

In summary, data from a controlled dietary study were used to
analyze the cyclic fluctuation of the carotenoids in lipoprotein
fractions in premenopausal women. Significant cyclic changes
appeared in the concentration of each carotenoid in the LDL
fraction, except for lycopene. Approximately 80% of the hydro-
carbon carotenoids and 64% of lutein/zeaxanthin were distrib-
uted in the LDL fraction. The percentage of a carotenoid in the
LDL fraction remained fairly constant over the cycle whereas the
percentage in the HDL subfractions varied slightly. Plasma
carotenoid and lipoprotein concentrations fluctuated by phase of
the cycle (23, 24). Therefore, future research in premenopausal
women needs to take into consideration the dynamics of the
cyclic fluctuations of individual plasma carotenoids and their
concentrations in the lipoprotein carriers. These findings have
implications for ovarian function and may lead to the identifica-
tion of specific carotenoid-hormone relations.

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